**Rabbit antithymocyte globulin and donor-specific antibodies in kidney transplantation – a review**

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**Abbreviations**

| | |
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| ABMR | antibody-mediated rejection |
| BPAR | biopsy-proven acute rejection |
| CDC | complement-dependent cytotoxicity |
| CMV | cytomegalovirus |
| DSA | donor specific antibodies |
| dnDSA | de novo donor specific antibodies |
| HLA | human leukocyte antigen |
| IL-2RA | interleukin 2 receptor antagonist |
| IVIG | intravenous immunoglobulin |
| MMF | mycophenolate mofetil |
| NK | natural killer |
| PRA | panel reactive antibodies |
| PTLD | post-transplant lymphoproliferative disease |
| rATG | rabbit antithymocyte globulin |
| TCMR | T-cell mediated rejection |
| $T_{regs}$ | T-regulatory cells |

**Abstract**

The mode of action of rabbit antithymocyte globulin (rATG) includes preferential inhibition of pre-existing donor-reactive memory T-cell reconstitution and possibly apoptosis of plasma cells, the source of donor specific antibodies (DSA). In kidney transplant patients with low-strength preformed DSA, non-comparative data have shown a low incidence of antibody-mediated rejection (ABMR) and graft survival using rATG even without desensitization procedures. For high strengths of preformed DSA, rATG induction with more aggressive desensitization appears effective, with mixed results concerning the addition of B-cell specific agents. Regarding production of *de novo* DSA (dnDSA), interpretation of retrospective analyses is limited by selective use of rATG in higher-risk patients. Observational data in moderately sensitized kidney transplant patients suggest that the incidence of dnDSA and ABMR is significantly lower with rATG versus basiliximab. A randomized pilot study has suggested that addition of rituximab or bortezomib may not further inhibit dnDSA production in rATG-treated patients. Overall, rATG appears to inhibit DSA production, with a potential role in reducing the risk of ABMR in kidney transplant patients with high-strength preformed DSA, or lowering dnDSA in moderately sensitized patients. Randomized trials are awaited.

*Keywords:* rATG, rabbit antithymocyte globulin, Thymoglobulin, DSA, donor specific antibodies, antibody mediated rejection

## Introduction

The poor prognosis associated with anti-human leukocyte antigen (HLA) donor-specific antibodies (DSA) following kidney transplantation is well-established. Preformed class I and II DSA, in particular, confer a marked increase in the risk of antibody-mediated rejection (ABMR) [1-3] and reduced allograft survival [1, 2, 4, 5], even when the titer is below the threshold for a positive crossmatch, whereas preformed complement (C1q)-fixing DSA shows a less convincing association with poor outcomes [6, 7]. Development of *de novo* DSA (dnDSA) after kidney transplantation also incurs a higher risk for acute rejection [8, 9], chronic ABMR [10] and graft survival [4, 10, 11]. Complement-binding dnDSAs show a particularly strong association with ABMR and graft failure, increasing the risk of graft loss by over four-fold [10]. Rates of acute T-cell mediated rejection (TCMR) and ABMR are both higher in kidney transplant patients who develop dnDSA compared to recipients with preformed DSA [12], and the combination of 'mixed' TCMR and ABMR is especially unfavorable. Of note, donor-specificity of HLA antibodies is highly important; HLA antibodies that are not donor-specific appear to be less relevant [1].

There is no conclusive evidence to confirm that any immunosuppressive regimen or agent prevents or delays DSA production. However, randomized clinical trials, undertaken before routine DSA monitoring was adopted, have pointed to a possible effect for rabbit antithymocyte globulin (rATG) induction. Randomized studies have shown rATG to be effective in preventing biopsy-proven acute rejection (BPAR), and specifically, steroid-resistant BPAR, in kidney transplant patients categorized as sensitized based on anti-HLA panel reactive antibody (PRA) status or other established risk factors [13–15]. An early trial of 89 patients with PRA in the range 5–100%, with or without positive complement-dependent cytotoxicity (CDC) B-cell crossmatch, showed

that compared to no induction, rATG induction significantly reduced BPAR and increased one-year graft survival and function, even at the highest levels of sensitization (PRA >80%) [15]. Rates of ABMR were not reported. More recently, a randomized trial comparing rATG induction versus the interleukin 2 receptor antagonist (IL-2RA) daclizumab in 227 HLA-sensitized kidney transplant patients (current PRA ≥30% and/or peak PRA ≥50%) receiving tacrolimus, mycophenolate mofetil (MMF) and steroids as maintenance therapy showed a significant reduction rate of BPAR and steroid-resistant BPAR in the rATG-treated cohort at one year [14]. There was no difference in the rate of ABMR (one case occurred in each treatment arm), but interestingly only two rATG patients were given intravenous immunoglobulin (IVIG) and/or plasmapheresis (with another given OKT3), while in the daclizumab arm six patients were given IVIG, plasmapheresis or rituximab, and a further seven needed anti-rejection treatment with rATG. Brennan *et al* also reported a significant benefit for rATG versus IL-2RA induction in terms of BPAR and steroid-resistant rejection in another cohort of kidney transplant patients at increased risk for acute rejection or delayed graft function [13]. A systematic review with a meta-analysis has confirmed that when IL-2RA induction (basiliximab or daclizumab) is compared to ATG (16 randomized controlled trials, 2,211 participants), there is a benefit for ATG therapy over IL-2RA in terms of BPAR at one year, but at the cost of an increase in malignancy and cytomegalovirus (CMV) disease [16]. However, the meta-analysis included studies from the 1990s and early 2000s when ATG doses were markedly higher than at present, and also included several studies of equine ATG, so applicability to rATG induction with modern regimens is not certain. More recent registry analyses have shown mixed findings in terms of risk for post-transplant lymphoproliferative disease (PTLD) or malignancy, but again can be difficult to interpret since they were not necessarily specific to rATG [17–20]. In the TAILOR registry of living-donor kidney transplant recipients, 2,322 patients transplanted in 2003–2008 and

given a mean cumulative rATG dose of ~5.3mg/kg showed a PTLD incidence of 0.9% at five years, comparable with the kidney transplant population overall [20]. These data are a reminder that the overall intensity of immunosuppression should not be disproportionately increased, to avoid a heightened risk of malignancies and infections.

While these trials do not provide direct evidence regarding an influence of rATG on pre-existing DSA or the development of dnDSA, they do suggest that use of rATG induction merits further exploration to examine the balance of benefits and risks. The current data relating to rATG (Thymoglobulin) and anti-HLA DSA are discussed here.

**The mode of action for rATG: potential relevance to DSA production**

ABMR is a progressive process, diagnosed based on the presence of circulating DSA with specific histologic criteria (primarily microvascular inflammation and transplant glomerulopathy) and immunohistologic characteristics [21, 22].

rATG interacts with a large range of antigens on immune and non-immune cell types, inducing apoptosis of B-cells, peripheral T-cells and natural killer (NK) cells, and modulates leukocyte/endothelium interactions [23–25]. Evidence from a murine model has shown that rATG targets pre-existing donor-reactive memory T-cells, suppressing their recovery more effectively than other components of the T-cell response [26]. In addition, the well-documented phenomenon of preferential reconstitution of T-regulatory cells ($T_{regs}$) after rATG treatment [27–29] may also be beneficial.

rATG may also exert a direct effect, since it contains antibodies against several plasma cell antigens. *In vitro* studies by Zand *et al* have shown that rATG strongly induces apoptosis in terminally differentiated plasma cells ($CD138^+$) at clinically relevant

concentrations (1–100ng/mL) via a complement-independent process [30] and may thus potentially inhibit production of DSA, although this has not been demonstrated in these studies. Other researchers, however, have observed no effect of rATG (or rituximab or IVIG) on plasma cell apoptosis *in vitro* [31] or *in vivo* after desensitization with rATG [32], although $CD27^+$ memory B-cells appear to be depleted [32].

Taken together, from the complex impact of rATG on blood cell constituents, especially on the plasma and $T_{reg}$ compartment, it could be hypothesized that rATG also affects DSA production post-transplant and the risk for ABMR. However, this remains to be evaluated.

**rATG in presensitized patients**

Anti-HLA antibodies have been detected in 10–24% of patients prior to kidney transplantation [33–35], with estimates influenced by the choice of techniques and the era of the study population. Organ matching is challenging in broadly sensitized patients due to the high immunologic barrier. Even if transplantation is performed and crossmatches are negative, presensitization with DSA predicts poor graft survival [10, 34, 36–38]. Survival is especially low when DSA persist [10] or increase [39] post-transplant, due to higher rates of ABMR [36, 39]. Known risk factors for presensitization against HLA antigens include prior blood transfusions [40, 41], pregnancy [40], and previous surgery including prior transplantation [42]. Infectious agents may also potentiate an anti-HLA response as a result of molecular mimicry [43].

Desensitization protocols are complex, but the most widely used downregulation strategies are plasmapheresis and/or IVIG, frequently with intravenous administration of the chimeric monoclonal anti-CD20 antibody rituximab. There has also been recent

interest in the plasma cell-targeted protease inhibitor bortezomib and the anti-complement antibody eculizumab [44]. These regimens have acceptable short-term graft survival, but rates of acute rejection and ABMR remain much higher than in non-sensitized patients [45]. Encouragingly, however, a large cohort study of 211 live donor kidney recipients reported a significant survival benefit following desensitization versus remaining on dialysis [46].

Numerous studies have reported outcomes using different preconditioning regimens and rATG induction, as discussed below. With no consensus regarding the optimal combination and doses of desensitizing techniques, these studies describe a wide range of populations and methodologies. No trial has compared outcomes following a preconditioning regimen with or without the use of rATG induction, limiting an accurate assessment of the specific contribution of rATG in any regimen.

– *Low-strength DSA*

The risk of ABMR increases with DSA strength at the time of transplantation [47]. Nevertheless, in candidates with low-strength DSA (i.e. DSA detectable only on more sensitive assays such as flow-cytometric crossmatch or single antigen flow beads), ABMR rates are still higher than in DSA-negative patients despite relatively weak sensitization. Without preconditioning, acute and chronic ABMR has been reported in 33% and 42% of these patients [48], although graft survival rates are typically similar to patients with negative crossmatch [47]. No study has compared outcomes using rATG induction or no rATG induction in kidney transplant patients with low-strength DSA. Two centers have described the results of desensitization in a population of kidney transplant recipients with low-strength DSA who received rATG induction, with no control regimen [49, 50] (Table 1). Bächler and colleagues prospectively identified the presence of low-

strength DSA by single antigen flow beads ('virtual crossmatch') in 37 candidates (all with negative T-cell and B-cell CDC crossmatch) who received IVIG prior to graft reperfusion and on days 1–4 (total dose 2g/kg), with rATG (using the Fresenius preparation, not Thymoglobulin®) 9mg/kg prior to reperfusion and 3mg/kg on days 1–4 [49]. Maintenance immunosuppression comprised tacrolimus, MMF and steroids. Compared to a cohort of 67 historical controls, also with low-strength DSA but without additional treatment with IVIG or rATG (but with IL-2 receptor blockade in 48%), the rate of ABMR in clinically-indicated biopsies was markedly lower six months after transplantation in the IVIG/rATG treatment group (11% versus 46%, p=0.0002). In addition, the rate of TCMR in indication biopsies was also significantly lower in the IVIG/rATG-treated patients (0% versus 50%, p<0.0001). Subclinical TCMR on protocol biopsies at three and six months was also less frequent in the IVIG/rATG cohort (11–18% versus 43–46%, p=0.008-0.03) [49]. The rate of subclinical ABMR did not differ between the groups. Akalin *et al* identified a subgroup of patients who were CDC crossmatch T-cell negative but B-cell positive, or flow cytometry crossmatch positive, and stratified them according to DSA strength on single antigen flow bead assay [57] (Table 1). In the 12 patients with 'weak or moderate' DSA strength, IVIG with rATG induction (1.5mg/kg/day for five days) and a regimen of tacrolimus, MMF and steroids prevented graft rejection entirely, although the absence of a control group means that strong conclusions cannot be drawn. In contrast, other small retrospective studies comparing patients with low-strength DSA versus a DSA-free control group have suggested that ABMR and graft survival rates are similar even in the absence of desensitization procedures when using rATG induction with tacrolimus, MMF and steroids [51, 52].

– *High-strength DSA*

Compared to patients with low-strength DSA, patients with high-strength DSA are at significantly greater risk of ABMR, as well as both early and late graft loss [38, 47]. Stegall and colleagues used rATG induction in 61 patients with CDC T-cell positive crossmatch [55] (Table 1). The desensitization protocol evolved over the period of analysis, from (i) plasmapheresis with low-dose IVIG and rituximab (with splenectomy in the early cases), to (ii) high-dose IVIG switching to the earlier regimen for non-responders, then finally to (iii) plasmapheresis, low-dose IVIG and rituximab with pre-transplant rATG. rATG (1.5mg/kg/day) was given after plasmapheresis on the first five days of the preconditioning regimen with post-transplant DSA monitoring and further intervention was given as required to maintain a low DSA strength [55]. The latter regimen, with rATG induction, achieved a lower rate of ABMR than the early rituximab-containing protocol (29% versus 37%), although follow-up times are not reported. One study, by Vo *et al*, has compared rATG induction versus IL-2RA induction (daclizumab) in two sequential cohorts of CDC crossmatch-positive patients who received IVIG preconditioning [54]. In the earlier phase of this retrospective study, 58 patients received daclizumab; latterly, 39 patients were given rATG induction. The incidence of ABMR by two years was similar (daclizumab 21%, rATG 22%) [54]. Graft survival (84% and 90%, respectively) and patient survival (96% and 100%, respectively) were not significantly different (Table 1), but more daclizumab-treated patients were crossmatch-negative by the time of transplant (48% versus 25%, $p<0.03$), which is likely to have skewed the results [54]. Confirmatory trials are awaited.

Other centers have reported their experience with rATG induction after use of various preconditioning protocols, allocated according to the risk level of transplant candidates

based on T-cell immunoglobulin titers, positive T-cell and/or B-cell crossmatch by CDC, flow cytometry, ELISA or single antigen flow bead assay [58–60]. Some reports have administered rATG in an unidentified proportion of patients [46, 60, 61], making it difficult to identify the contribution of rATG. One randomized trial in 40 kidney transplant patients has compared rATG alone (9mg/kg total dose) to rATG (6–7.5mg/kg) with rituximab or with bortezomib, or with both agents [62]. The study population, however, included patients if they had high cytotoxic PRA levels or prior allograft loss with more than one rejection, as well as patients with confirmed DSA by CDC or flow cytometry, and no more than 6/10 patients in each treatment group had a confirmed positive crossmatch or DSA. Nevertheless, it is interesting that an increase in preformed DSA occurred in no patients given rATG alone, but in up to 40% of patients in the other groups. However, rATG with rituximab entirely prevented ABMR, whereas one patient (10%) given rATG alone had ABMR and three patients (30%) given rATG and bortezomib had ABMR, one of whom lost their graft as a result [62]. Addition of B-cell specific agents to rATG induction may be effective, but these findings are inconclusive and trials exclusively in recipients with pre-existing DSA are lacking. Moreover, it should be noted that one retrospective study of 77 kidney transplant patients given rituximab for various reasons showed a significantly higher rate of death from infectious causes than a control group of patients without rituximab therapy [63].

Overall, rATG induction for presensitized kidney transplant patients, even with low-strength DSA, appears to be an appropriate adjunct to the desensitization process. In patients with high strengths of pre-transplant DSA, the potential incremental benefit of rATG with additional preconditioning strategies remains unclear, and no firm conclusions can be drawn regarding the use of rATG in this setting.

***De novo* DSA**

Little is known about the dominant risk factors for dnDSA. Two proposed factors are poor HLA matching, particularly at the DQ locus [11, 64, 65], and non-adherence to the immunosuppressive regimen [66]. Younger patients (e.g. <50 years) are at increased risk [8, 67], possibly due to a more robust immunological response and greater non-adherence. African American recipients may also be prone to develop dnDSA [64]. Recent studies have reported an incidence of 8–11% by the end of the first year after kidney transplantation [68, 69], rising as high as 25% in patients at high immunological risk [62]. Although theoretically use of rATG induction therapy to inhibit activity of both T-cells and B-cells in the immediate post-transplant period would seem a rational strategy to reduce early dnDSA production in at-risk individuals [23], published data supporting this is particularly limited.

*rATG induction and dnDSA production*

Prospective data on the rate of dnDSA are available from a subpopulation analysis of 37 kidney transplant patients taking part in a randomized trial assessing early corticosteroid withdrawal, all of whom received rATG induction with tacrolimus and MMF maintenance therapy [70]. All patients showed a negative CDC crossmatch at baseline. Annual follow-up included mixed bead antigen testing, with single antigen flow bead testing in those who tested positive. By year 5, only one of the 37 patients (2.7%) had developed dnDSA, but this low rate may have reflected the study definition of dnDSA i.e. antibodies which developed after the first post-transplant year [70].

Retrospective analyses [4, 8, 12] have described the baseline characteristics –- including the type of induction therapy – in kidney transplant patients who did or did not develop dnDSA during follow-up, but interpretation is hampered by bias in the use of rATG induction. Huang *et al* administered rATG pre-transplant in all high-risk patients

(defined as preformed DSA, African American recipients, retransplants, or PRA >20%) and used basiliximab, rATG or no induction in low-risk patients [12] (Table 2). The observation that there was no significant difference in rATG use in patients with or without dnDSA (Table 3) is largely unhelpful given the selective nature of rATG administration (Table 3). Similarly, Cooper *et al* observed an identical proportion of rATG use in a series of 244 patients who did or did not develop dnDSA by month 24 post-transplant, but rATG was again used preferentially in recipients at higher immunologic risk [8]. Consistent with this, a retrospective analysis of 1,229 patients undergoing kidney transplant over an extended period (1972–2002) has reported the use of rATG to be higher in the subpopulation who developed dnDSA (Table 3) but the difference was lost on multivariate analysis, reflecting the selective use of rATG in patients at high risk (i.e. retransplant, PRA ≥15% or cold ischemia ≥36 hours) [4]. Kanter Berga *et al* undertook a cross-sectional analysis of dnDSA occurrence based on single antigen flow bead assay in 321 recipients of a kidney transplant at standard immunological risk, and observed the use of induction (either rATG or basiliximab) to be significantly lower in the patients who developed dnDSA versus those who remained non-sensitized or developed non-DSA HLA antibodies (22.2% versus 54.5% and 70%, p=0.02), but data were not provided separately for rATG and basiliximab [73].

One recent retrospective analysis has reviewed the development of dnDSA in 196 non-sensitized patients undergoing heart transplantation at a single center during 2006 to 2013 [74]. rATG induction was given at a dose of 1.5 mg/kg for 3–5 days in 35 patients, with no induction in the remaining patients. Maintenance therapy comprised tacrolimus, MMF and steroids across the entire population. At one year, the proportion of patients with *de novo* HLA antibody production was significantly lower in the subgroup treated with rATG (11% versus 21% in patients without induction, p=0.043) but dnDSA was

similar (9% versus 12%, p=0.541). Imbalances between the two groups, and the relatively short follow-up time, may have influenced the results.

- *Comparisons with other induction regimens*

A recent observational analysis has compared the incidence of dnDSA in 114 consecutive kidney transplant patients who received either rATG or basiliximab induction, both with tacrolimus, MMF and steroid maintenance therapy [71]. The patients were all moderately sensitized: inclusion criteria were negative crossmatch on flow cytometry but DSA-positive using single antigen flow bead testing (500 to 4,000 mean fluorescence intensity [MFI]). The desensitization protocol comprised plasmapheresis with IVIG, and rATG or basiliximab induction was given according to physician preference. As might be expected, the rATG group were at higher immunological risk, with significantly higher peak PRA (p=0.03), greater use of plasmapheresis/IVIG (p=0.0008) sessions, and more IVIG injections compared to the basiliximab-treated patients [71]. Despite this, after a follow-up of up to three years, rates of dnDSA and ABMR were both significantly lower in the rATG-treated cohort based on Kaplan-Meier analyses (Figure 1). Interestingly, the benefit for rATG appeared relatively late – after month 12. The benefits of rATG were confirmed in a stepwise multivariate regression analysis, which showed a hazard ratio (HR) of 0.16 for dnDSA and also for ABMR when using rATG versus basiliximab (Table 3). For those patients who did develop dnDSA, levels were strikingly lower with rATG (mean 455 MFI versus 3,652 with basiliximab; p=0.02). These results suggest that rATG induction achieves a decrease in dnDSA production in moderately sensitized patients over the first three years following kidney transplantation compared to IL-2RA induction [71].

A randomized prospective pilot study by Ejaz *et al* has compared rates of dnDSA between kidney transplant patients receiving rATG alone, or with the addition of rituximab, bortezomib or both rituximab and bortezomib (the number of rATG doses was reduced according to the concomitant therapy) [62]. The study population was at high risk, selected on the basis of PRA ≥20% (or historical PRA ≥50%), T-cell or B-cell positive crossmatch on flow cytometry, or positive CDC crossmatch with confirmed DSA, or loss of a previous graft to acute rejection. Maintenance immunosuppression comprised tacrolimus, MMF and steroids. At the end of the one-year study, there was no difference in the rates of dnDSA or ABMR between the four groups, although absolute numbers were low (Table 3). Based on these initial data, addition of profound naïve and memory B-cell depletion using rituximab, or plasma cell apoptosis via bortezomib, do not appear to further inhibit dnDSA production in sensitized kidney transplant patients given rATG induction, although further data are required. In another comparative analysis, Todeschini and colleagues undertook a retrospective study in which they compared lymphocyte reconstitution and dnDSA in 16 kidney transplant patients treated with alemtuzumab induction versus a matched cohort of 32 rATG-treated patients [72]. All patients were DSA-negative at time of transplant, but by year 1 the incidence of dnDSA was significantly lower in the rATG cohort (12.5% versus 50%, p=0.01), a difference the authors attributed to alemtuzumab-induced changes to B-cell phenotypes, notably an expansion of naïve B-cells [72].

**Conclusions**

There is currently intense interest in the prognostic importance of preformed DSA and newly formed DSA after organ transplantation, but undertaking trials to define the best immunosuppressive strategy to minimize the risks associated with DSA is challenging.

Controlled trials of desensitization techniques are limited by ethical considerations for a control arm and lack of agreement on suitable endpoints such as DSA titers or histological changes. Evaluation of prevention of dnDSA is less problematic, but is likely to be restricted to patients with at least moderate sensitization to avoid unnecessary intervention. Comparative studies of immunosuppressive agents are now starting to routinely include baseline assessments of anti-HLA DSA status, and to monitor DSA post-transplant, but in the meantime the transplant community is required to make prescribing decisions based on the available, imperfect evidence base.

Despite these caveats, rATG appears to inhibit DSA production. rATG induction may be helpful in reducing the risk of ABMR in presensitized kidney transplant patients with high-strength DSA. In patients with low-strength preformed DSA, regimens including rATG induction have been effective in avoiding ABMR and achieving good graft survival rates. However, comparative data are lacking which makes it difficult to draw conclusions. Consistent with results from randomized trials showing improved BPAR rates with rATG versus IL-2RA induction [13,14] in high immunological risk kidney transplant recipients and, in one trial, improved graft survival versus no induction [15], rATG induction appears to reduce the risk of dnDSA and ABMR in moderately sensitized patients compared to non-B-cell depleting IL2-RA induction therapy [71].

Future studies of rATG could usefully include a protocol-defined schedule for DSA monitoring to monitor DSA recurrence after desensitization and occurrence of *de novo* DSA, with longer-term follow-up.

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*Conflicts of interest*

Dr Julio Pascual has received honoraria from Sanofi. Andreas Zuckermann is a member of a speaker's bureau and advisory board for Sanofi, a member of a speaker's bureau for Novartis, and has received a scientific grant from Roche. Arjang Djamali has received travel support from Sanofi and grants from BMS and Takeda. Alexandre Hertig has received speaker's honoraria from Sanofi. Maarten Naesens is a member of advisory boards for Roche, Novartis and Sanofi, has received speaker's honoraria from Novartis, Astellas and Shire, and has received travel support from Sanofi, Novartis and Astellas.

*Author contributions*

All authors contributed to the manuscript development, providing critical review and final approval of the manuscript for submission.

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**Figure legend**

**Figure 1.** (a) De novo donor specific antibodies [dnDSA] and (b) antibody-mediated rejection [ABMR] during the first three years after kidney transplantation in 114 moderately sensitized patients in an observational study (Kaplan-Meier estimates). Adapted with permission from Reference 71

**Table 1**. rATG induction in patients receiving desensitizing regimens prior to kidney transplantation

| Study | Design/ donor type | n | Crossmatch detec method | Sub-groups | Desensitization | Induction/ maintenance immuno-suppression | Follow-up | AMBR | Graft survival | Patient survival |
|---|---|---|---|---|---|---|---|---|---|---|
| *Low-strength DSA* | | | | | | | | | | |
| Knight 2013 [51] | Retrospective Living donor | 44 | CDC + flow cytometry | CDC XM-negative FC XM-negative | None | rATG TAC MMF Steroids | Median 26 months | 3.3% | 100% | - |
| | | | | CDC XM-negative FC XM-positive | | | | 0% | 100% | - |
| Bächler 2010 [49] | Prospective Historical controls Deceased or living donor | 37 (+ 67 controls) | CDC + SAFB | Prospective cohort, SAFB XM-positive | IViG | rATG TAC MMF Steroids | Median 2 years | 11% at month 6 | 0% due to ABMR at year 1 | - |
| | | | | Controls, SAFB XM-positive | None | No rATG Various | Median 8.5 years | 46% at month 6 (p=0.0002) | 7.5% due to ABMR at year 1 | |
| Roberti 2007 [52] | Retrospective Pediatric patients Living donor | 50 | CDC, flow cytometry, SAFB | CDC XM-negative FC XM-negative SAFB XM-negative | None | rATG TAC MMF Steroid | 3 years | 0% | 100% | 96.7% |
| | | | | CDC XM-negative FC XM-negative SAFB XM-positive | | Basiliximab TAC MMF Steroids | 3 years | 0% | 100% | 91% |
| Thielke 2005 [50] | Retrospective Living donors | 16 | CDC + flow cytometry | - | PP IVIg | rATG TAC MMF Steroids | | 25% | 100% | 100% |
| *High DSA* | | | | | | | | | | |
| Zhang 2011 [53] | Retrospective Living donor | 14 | CDC, flow cytometry, SAFB | - | PP IVIG ±rituximab | rATG TAC MMF Steroids | 1 year | 14.3% | 92.9% | 100% |
| Vo 2006 [54] | Retrospective 2 subgroups (by induction type) | 97 | CDC | rATG induction | IVIG | TAC MMF Steroids | 2 years | 21[a] | 90[a] | 100[a] |
| | | | | Daclizumab | | | | 22[a] | 84[a] | 96[a] |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Deceased or living donor | | | induction | | | | | | |
| Stegall 2006 [55] | 3 subgroups (by treatment) | 61 | CDC, flow cytometry, single- antigen flow beads | Sequential desensitizing protocols | PP<br>Low-dose IVIG<br>Rituximab<br>±splenectomy | rATG<br>TAC<br>MMF<br>Steroids | n/a | 37% | - | - |
| | | | | | High-dose IVIG | | | 80% | - | - |
| | | | | | PP<br>Low-dose IVIG<br>Rituximab<br>Pre-tx rATG (5 days) + Post-tx DSA monitoring | | | 29% | - | - |
| *Various DSA levels* | | | | | | | | | | |
| Mai 2009 [56] | Retrospective<br>3 subgroups (by PRA) | 94 | Flow cytometry | PRA<20%, FC-XM negative | none | rATG<br>TAC<br>MMF | 3 years | 1.7 | 78.6% | 90.6% |
| | Deceased or living donor | | | PRA >20%, FC XM-negative | none | Steroids | | 6.3 | 80.4% | 93.8% |
| | | | | PRA >20%, FC XM-positive | IVIG | | | 30.0 | 88.7% | 93.8% |
| Akalin 2008 [57] | Prospective<br>3 subgroups (by DSA)<br>Living donors | 35 | CDC T-cell – negative and CDC B-cell-positive or FC-positive | Low/moderate DSA | IVIG | rATG<br>TAC | Median 18 | 0% | 100 | 100% |
| | | | | High DSA | IVIG | MMF<br>Steroids | | 44% | 78 | 100% |
| | | | | High DSA | IVIG + PP | | | 7% | 86 | 93% |

CDC, complement-dependent cytotoxicity; DSA, donor-specific human leukocyte antibodies; FC, flow cytometry; n/a, not available; IVIG, intravenous immunoglobulin; MMF, mycophenolate mofetil; PP, plasmapheresis; rATG, rabbit antithymocyte globulin; SAFB, single-antigen flow beads; PRA, panel reactive antibodies; TAC, tacrolimus; XM, crossmatch

[a] Fewer primary transplants ($p<0.002$) and fewer patients achieving negative crossmatch ($p<0.03$) by time of transplant in the rATG group versus the daclizumab group

Low-strength DSA defined as negative CDC crossmatch with DSA detectable by flow cytometry or single-antigen flow beads. High-strength DSA defined as positive CDC T-cell or B-cell crossmatch

**Table 2.** rATG induction therapy in patients with or without dnDSA

| Study | Study type/ Time period / Donor type | n | Crossmatch detection method | Induction/ maintenance immuno-suppression | Follow-up | Induction type | % rATG in DSA+ patients | % rATG in DSA- patients | P value |
|---|---|---|---|---|---|---|---|---|---|
| Huang 2012 [12] | Retrospective 2010-2011 Kidney or kidney-pancreas | 173 | FC, mixed antigen flow beads + SAFB | CsA or TAC MMF Steroids | 480 days | rATG[a] | 80 | 67 | 0.30 |
| | | | | | | BAS[a] | 0 | 12 | |
| | | | | | | No induction[a] | 20 | 21 | |
| Kanter Berga 2011 [33] | Retrospective 1997-2009 Deceased-donor kidney | 321 | CDC, SAFB | CsA or TAC (otherwise not specified) | Mean 62 months | rATG or BAS | 22.2 | 54.5[b] | 0.02 |
| | | | | | | No induction | | | |
| Cooper 2011 [8] | Retrospective 2007-2009 Kidney or kidney-pancreas | 244 | FC + mixed antigen flow beads | TAC[a] MMF[a] Steroids[a] | 2 years | rATG | 66 | 66 | 0.73 |
| | | | | | | BAS | 6 | 4 | |
| | | | | | | No induction | 28 | 30 | |
| Hourmant 2005 [4] | Retrospective 1972-2002 Kidney or kidney-pancreas | 1229 | ELISA, CDC and/or SAFB | Mixed | 5 years | rATG | 72[b] | 58 | <0.001[c] |

[a] 87% of patients received TAC/MMF/steroids
[b] 70% in patients with non-donor specific HLA antibodies
[c] 78% in patients with non-donor specific HLA antibodies
[d] Significance was lost on multivariate analysis
BAS, basiliximab; CDC, complement-dependent cytotoxicity; CsA, cyclosporine; DSA, donor-specific human leukocyte antibodies; FC, flow cytometry; MMF, mycophenolate mofetil; rATG, rabbit antithymocyte globulin; SAFB, single-antigen flow beads; TAC, tacrolimus

**Table 3.** dnDSA production in rATG-treated transplant patients

| Study | Study type/ Time period / Donor type | n | Crossmatch detection method | Induction/ maintenance immuno-suppression | Follow-up | Induction type | % dnDSA at last follow-up | | % ABMR at last follow-up | |
|---|---|---|---|---|---|---|---|---|---|---|
| Brokhof 2014 [71] | Observational 2009-2011 Deceased-donor kidney | 114 | SAFB | TAC MMF Steroids | 3 years | rATG | HR 0.16, 95% CI 0.04-1.50, p=0.003 for rATG vs BAS[a] | | HR 0.16, 95% CI 0.05-0.60, p=0.006 for rATG vs BAS[a] | |
| | | | | | | BAS | | | | |
| Ejaz 2013 [62] | Prospective Randomized 2008-2013 Kidney | 40 | CDC, FC, SAFB | TAC MMF Steroids | 1 year | rATG | 30% | p=0.70 | 10% | p=0.36 |
| | | | | | | rATG + rituximab | 30% | | 0% | |
| | | | | | | rATG + bortezomib | 10% | | 30% | |
| | | | | | | rATG + rituximab + bortezomib | 30% | | 10% | |
| Todeschini 2013 [72] | Retrospective Matched cohort Kidney | 48 | Mixed antigen flow beads | Low-dose sirolimus or low-dose CsA MMF Steroids to day 7 | 2 years | Low-dose rATG + BAS | 12.5% | p=0.011 | - | |
| | | | | | | Alemtuz-umab | 50% | | - | |
| Delgado 2009 [70] | Prospective First kidney transplant | 37 | CDC, mixed antigen flow beads + SAFB | TAC MMF ± Steroids | ≤5 years | rATG + steroids | 6.3% | | - | |
| | | | | | | rATG no steroids | 0% | | - | |

ABMR, antibody-mediated rejection; BAS, basiliximab; CDC, complement-dependent cytotoxicity; CI, confidence interval; CsA, cyclosporine; dnDSA, de novo donor-specific human leukocyte antibodies; FC, flow cytometry; n/a, not available; HR, hazard ratio; MMF, mycophenolate mofetil; rATG, rabbit antithymocyte globulin; SAFB, single-antigen flow beads; TAC, tacrolimus

[a] Multivariate analysis